Abstract No. maro341

Biolochemistry of Nickel

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Beamline(s): X9B

ABSTRACT: Acireductone dioxygenase ARD is a 20.2 kDa enzyme involved in the methionine salvage pathway, and is the first example of a Ni-containing dioxygenase. This pathway provides regulation of S-methylthioadenosine (MTA), a strong inhibitor of polyamine biosynthesis and transmethylation reactions. The methionine salvage pathway regulates MTA by converting its 5-methylthio-D-ribose portion to methionine. Two enzymes catalyzing distinct reactions of acireductone substrates were identified in *Klebsiela pneumoniae* (see scheme). These enzymes were found to contain the same polypeptide chain, but differ in the metal center in the active site. ARD, which makes CO and an acid that is one C atom shorter than that produced by ARD', is a Ni enzyme, while ARD' is an Fe enzyme. The gene has been cloned and the protein expressed in *E. coli*. The structure of the folded polypeptide has been determined by NMR in the Pochapsky lab, except for the region around the paramagnetic metal centers. This study shows that the two Cys residues in the enzyme are close to the metal site. Preliminary XAS measurements show that the Ni site in resting ARD (oxidized) is six coordinate with O/N donor ligands, at least one of which is a His ligand from multiple scattering analysis of the EXAFS data. There is no evidence of an S-donor ligand. Such an environment would not be expected to support Ni redox chemistry. Therefore, a mechanism involving substrate activation to attack by O_2 is favored over a mechanism involving O_2 activation via reduction at the Ni center.

Preliminary results indicate that there are no free thiols in the resting (oxidized) enzyme, so the two Cys residues form a disulfide that is not a Ni ligand. Future experiments will address the role of the disulfide in the reductive activation of the enzyme. Does the active (reduced) enzyme have a Ni-S-Cys ligand (or two)? By eliminating O_2 from the sample, we will examine the structure of the Ni site in the enzyme/substrate complex to determine if the substrate binds to the Ni center. By comparing similar XAS data from the Fe site in ARD', we will be able to determine if the Fe center has the same ligand set as the Ni center in ARD.